Gene Expression and Host Biomarkers for Predicting Mortality in Melioidosis

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INTRODUCTION

Melioidosis is an infectious disease caused by Burkholderia pseudomallei (*B. pseudomallei*), a gram- negative bacteria and bio threat agent (Mukhopadhyay, Shaw, Varghese, & Dance, 2018). This disease is commonly found in parts of southeast Asia and northern Australia (Chin, Monack, & Nathan, 2010). *B. pseudomallei* is a facultative intracellular pathogen (Currie, 2015). *B. pseudomallei* is soil associated and can be found in muddy areas such as rice paddy fields (Krishnananthasivam et al., 2017). The rainy season contributes to the higher rates of the disease (Lazar Adler et al., 2009). Although the bacteria may be localized, it can be spread to organs (Lazar Adler et al., 2009). Melioidosis has a mortality rate of around 35% (Hinjoy et al., 2018). The main way of becoming infected is through inoculation of the skin but other routes include inhalation of aerosolized bacteria or ingestion of contaminated water (Krishnananthasivam et al., 2017). Melioidosis can cause an acute or chronic infection that may involve only one organ or involve many organs progressing to septic shock (Chin et al., 2010). Septic shock could be related to the spread of the bacteria, high mortality, and pneumonia (Wiersinga et al., 2007). Comorbidities such as immunosuppression, diabetes mellitus, and chronic kidney disease are greatly associated with Melioidosis (Krishnananthasivam et al., 2017). The disease also can remain dormant for months and even years thus causing the person to relapse (Chin et al., 2010).

OBJECTIVE

The objective of this study is to validate the gene expression markers in blood of survivors vs. non-survivors with melioidosis that may be used for monitoring treatment response in melioidosis. By looking at the gene alterations, they may be used as host-based biomarkers for predicting outcomes and diagnosing melioidosis. By using the gene expression markers in the blood, treatment response in melioidosis may be monitored. In this transcriptomic study, whole blood from non-survivors and survivors of melioidosis were used to analyze and compare several differentially expressed genes Functional analysis was conducted to show the major differentially expressed genes that are involved with the immune system's biological processes. The innate and adaptive immunity signaling pathways are important in driving outcomes of melioidosis patients.

MATERIALS AND METHODS

To validate the gene expression of these markers, the total RNA was extracted from the whole blood of patients at five hospitals in Northeast Thailand. Blood samples were collected from 19 non-survivor and 17 survivor patients with melioidosis. The RNA was then converted to cDNA. In this study, five genes involved in immune system pathways *TLR2, TLR4, PFKB3, RASGRP*, and *CD160* were selected. Three housekeeping genes *CycloA, TBP*, and *HPRT* were also used for data analysis. The gene expression profiles of each of the chosen immune signaling genes along with the total RNA were then analyzed using a reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). The data was analyzed using the Mann Whitney Test, P-values of < 0.05 were significant.

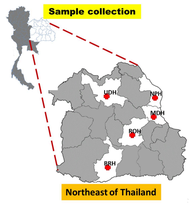

Figure 1: Location of hospitals in Northeast Thailand where samples were collected

TLR2 and TLR4 could possibly be used as biomarkers for predicting melioidosis mortality

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RESULTS

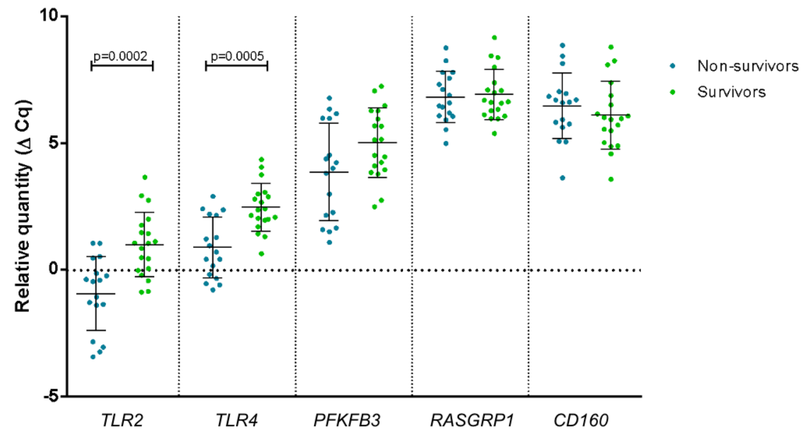

Figure 2: Relative quantity of expression of genes involved in the immune pathway in melioidosis patients with the status of non-survivors compared to survivors.

CONCLUSIONS

A total of 36 patient samples were analyzed in this study of which 17 were classified as non-survivors and 19 were classified as survivors. Five genes were manually selected from the immune pathway analysis; three genes were upregulated in non-survivors and two genes were downregulated in non-survivors when compared to survivors. Using three housekeeping genes HuP0, TBP, and HPRT were used to normalize the Cq values and get the relative quantity. After comparing non-survivors and survivors using the Mann-Whitney U Test, only two genes were significant (p value < 0.05). TLR2 and TLR4 were more highly expressed in non-survivors compared to survivors. PFKFB3, RASGRP1, and CD160 were not significant.

FUTURE DIRECTIONS

This study was not to diagnose melioidosis but to build models to predict early mortality in melioidosis. In this study, the sample size and number of genes selected were very small. Only two genes were significant out of the 5 selected. In future studies, an increased sample size and a wider range of genes will be used. Five genes are not enough to answer the question so more must be observed. By looking at genes such as these, they could predict and detect sepsis. Drugs may be developed to target and decrease the expression of these genes and reduce the possibility of patient mortality.

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